type (ST) 354. Isolates PET01 and PET05, identified from cats, belonged to ST93 and a new ST strain, respectively. Isolates EC08 and EC09, from the patients who shared the same hospital room with the pet shop worker, were ST156 (Table). Results of pulsed-field gel electrophoresis were consistent with multilocus sequence typing results and showed that isolates consisted of 5 types (types I to V; online Technical Appendix, http://wwwnc.cdc.gov/EID/article/22/9/16-0464-Techapp1.pdf). Isolate EC07 was clonally related to 4 *E. coli* strains from dogs, according criteria described by Tenover et al. (*10*), suggesting possible transmission of *mcr-1*–harboring *E. coli* between dogs and the patient. Colistin resistance was successfully transferred to *E. coli* C600 through conjugation in all isolates, suggesting that *mcr-1* was located on transferable plasmids.

These findings suggest that *mcr-1*–producing *E. coli* can colonize companion animals and be transferred between companion animals and humans. The findings also suggest that, in addition to food animals and humans, companion animals can serve as a reservoir of colistin-resistant *E. coli*, adding another layer of complexity to the rapidly evolving epidemiology of plasmid-mediated colistin resistance in the community.

References

1. Liu Y-Y, Wang Y, Walsh TR, Yi L-X, Zhang R, Spencer J, et al. Emergence of plasmid-mediated colistin resistance mechanism MCR-1 in animals and human beings in China: a microbiological and molecular biological study. Lancet Infect Dis. 2016;16:161–8. http://dx.doi.org/10.1016/S1473-3099(15)00424-7
2. Nordmann P, Lienhard R, Kieffer N, Clerc O, Poirel L. Plasmid-mediated colistin-resistant *Escherichia coli* in bacteremia in Switzerland. Clin Infect Dis. 2016 Mar 1:ciw124. http://dx.doi.org/10.1093/cid/ciw124
3. Falgenhauer L, Waezsada SE, Yao Y, Imirzalioglu C, Käsbohrer A, Roesler U, et al. Colistin resistance gene *mcr-1* in extended-spectrum β-lactamase–producing and carbapenemase-producing Gram-negative bacteria in Germany. Lancet Infect Dis. 2016;16:282–3. http://dx.doi.org/10.1016/S1473-3099(16)00009-8
4. Tse H, Yuen KY. Dissemination of the *mcr-1* colistin resistance gene. Lancet Infect Dis. 2016;16:145–6. http://dx.doi.org/10.1016/S1473-3099(15)00532-0
5. Malhotra-Kumar S, Xavier BB, Das AJ, Lammens C, Butaye P, Goossens H. Colistin resistance gene *mcr-1* harboured on a multidrug resistant plasmid. Lancet Infect Dis. 2016;16:283–4. http://dx.doi.org/10.1016/S1473-3099(16)00012-8
6. Tian GB, Huang YM, Fang ZL, Qing Y, Zhang XF, Huang X. CTX-M-137, a hybrid of CTX-M-14-like and CTX-M-15–like β-lactamases identified in an *Escherichia coli* clinical isolate. J Antimicrob Chemother. 2014;69:2081–5. http://dx.doi.org/10.1093/jac/dku126
7. Yong D, Toleman MA, Giske CG, Cho HS, Sundman K, Lee K, et al. Characterization of a new metallo-beta-lactamase gene, *bla*$_{NDM-1}$, and a novel erythromycin esterase gene carried on a unique genetic structure in *Klebsiella pneumoniae* sequence type 14 from India. Antimicrob Agents Chemother. 2009;53:5046–54. http://dx.doi.org/10.1128/AAC.00774-09
8. Wirth T, Falush D, Lan R, Colles F, Mensa P, Wieler LH, et al. Sex and virulence in *Escherichia coli*: an evolutionary perspective. Mol Microbiol. 2006;60:1136–51. http://dx.doi.org/10.1111/j.1365-2958.2006.05172.x
9. Tian GB, Wang HN, Zhang AY, Zhang Y, Fan WQ, Xu CW, et al. Detection of clinically important β-lactamases in commensal *Escherichia coli* of human and swine origin in western China. J Med Microbiol. 2012;61:233–8. http://dx.doi.org/10.1099/jmm.0.036806-0
10. Tenover FC, Arbeit RD, Goering RV, Mickelsen PA, Murray BE, Persing DH, et al. Interpreting chromosomal DNA restriction patterns produced by pulsed-field gel electrophoresis: criteria for bacterial strain typing. J Clin Microbiol. 1995;33:2233–9.

Acetobacter indonesiensis Bacteremia in Child with Metachromatic Leukodystrophy

Rebekka Kohlmann, Karin Barenberg, Agnes Anders, Sören G. Gatermann

Author affiliations: Ruhr-Universität Bochum, Bochum, Germany (R. Kohlmann, A. Anders, S.G. Gatermann); Institute of Medical Laboratory Diagnostics Bochum, Bochum (R. Kohlmann, S.G. Gatermann); Marienhospital Herne, Herne, Germany (K. Barenberg)

To the Editor: *Acetobacter indonesiensis*, first described in 2000 (*1*), belongs to the group of acetic acid bacteria (AAB), which includes the genera *Acetobacter*, *Gluconobacter*, *Asaia*, *Granulibacter*, and others in the family *Acetobacteriaceae*. AAB are of great industrial interest for use in vinegar fermentation processes because they oxidize alcohols or sugars incompletely, which leads

to acetic acid accumulation (*2*). AAB are widespread in nature and can be isolated from various sources, including vinegar, alcoholic beverages, tropical fruits, and flowers (*1,2*). AAB have rarely been associated with human disease. We describe a case of *A. indonesiensis* bacteremia in a child in Germany.

A 9-year-old girl with late-infantile metachromatic leukodystrophy was admitted to Marienhospital Herne, Herne, Germany, on February 9, 2015, for elective fundoplication. Because of her advanced neurologic disability, she required extensive nursing care and had several invasive medical devices, including a port catheter (detailed patient data in online Technical Appendix, http://wwwnc.cdc.gov/EID/article/22/9/16-0566-Techapp1.pdf). Soon after her admission, fever developed, and C-reactive protein levels increased (online Technical Appendix Table 1). Physical examination and further diagnostic investigations (e.g., chest radiograph) revealed no focus of infection. Because she had experienced recurrent pneumonia and candidemia (the latter led to port catheter exchange 1 month previously), antimicrobial drug treatment with piperacillin/tazobactam and caspofungin was administered.

One blood culture, drawn from the port catheter on day 3 of the hospital stay, yielded slowly growing multidrug-resistant bacteria (agar diffusion indicated zones of inhibition only for imipenem, meropenem, fosfomycin, and tigecycline). By partial sequencing of the 16S rRNA gene, we identified the isolate as *A. indonesiensis*. Details of microbiological analyses, colony morphologic features, 16S rDNA-based phylogenetic analysis, and antimicrobial drug susceptibility testing results are given in the online Technical Appendix.

Because the patient clinically responded to piperacillin/tazobactam and caspofungin treatment, therapy was continued for 15 days, although piperacillin/tazobactam showed no in vitro activity against the *A. indonesiensis* isolate. Despite the patient's improved condition, 1 control blood culture drawn from the port on hospital day 10, while she was receiving antimicrobial treatment, yielded *A. indonesiensis*, although another blood culture drawn peripherally on hospital day 14 yielded no growth. Port catheter exchange was advised but was not performed, according to the parents' wishes.

The first report of human infection with AAB can be traced to 2004, when peritonitis, associated with *Asaia bogorensis*, was reported in a peritoneal dialysis patient (*3*). Further reports include a description of *Granulibacter bethesdensis* as a cause of lymphadenitis in patients with chronic granulomatous disease (*4*), isolation of *Gluconobacter* spp. from a culture of blood from an intravenous drug user and of *Gluconobacter* spp. and *Asaia* spp. from sputum samples of cystic fibrosis

patients (*5*), a case of *A. bogorensis* bacteremia in an intravenous drug user (*6*), and central venous catheter–associated cases of *Asaia lannaensis* bacteremia in a child with cancer who had received a bone marrow transplant (*7*) and in children who had idiopathic dilated cardiomyopathy (*8*).

Regarding *Acetobacter* spp., only 2 reports on human infection have been published: *A. cibinongensis* bacteremia in a patient receiving chronic hemodialysis with signs of an infected arteriovenous fistula and suspected intravenous drug abuse (*9*) and *A. indonesiensis* pneumonia in a cystic fibrosis patient who was receiving immunosuppressive treatment because of a recent lung transplant (*10*). Similar to the case we report, species identification in those cases was achieved only with the help of sequencing methods in both cases. In the *A. indonesiensis* pneumonia case, results of antimicrobial drug susceptibility testing found that the bacteria showed multidrug resistance, as in the case we report, but susceptibility to aminoglycosides.

Of note, the aforementioned AAB infections all occurred in chronically ill patients or intravenous drug users. Similarly, children with metachromatic leukodystrophy are prone to healthcare- and device-associated infections involving opportunistic pathogens, and frequent use of broad-spectrum antibiotics may predispose the children for infection with multidrug-resistant bacteria. In the case we report, frequent accessing of the port, including for parenteral nutrition, may have further promoted microbial colonization.

Because a focus of infection was not apparent and because *A. indonesiensis* grew in 2 blood cultures independently drawn from the port but not in the blood culture obtained from peripheral venipuncture, we assume the patient's port catheter harbored the infectious agent. The fact that several previously reported AAB infections were catheter-associated may further support our suspicion. However, we could not confirm this assumption because the port was not removed and cultured.

The patient clinically responded to piperacillin/tazobactam and caspofungin treatment, despite a lack of in vitro activity against the *A. indonesiensis* isolate. Although this response might be explained by the presence of a second pathogen (which was not cultured but covered by the given antimicrobial agents), the control blood culture drawn from the port still yielded *A. indonesiensis* and at least argues for persistent colonization of the port. Because of pathogen persistence in blood culture and limited therapeutic options owing to the multidrug-resistance of the isolate, we believe the port should have been removed in this case.

Acknowledgments

Informed consent was obtained from the patient's parents for publication of this case report.

References

1. Lisdiyanti P, Kawasaki H, Seki T, Yamada Y, Uchimura T, Komagata K. Systematic study of the genus *Acetobacter* with descriptions of *Acetobacter indonesiensis* sp. nov., *Acetobacter tropicalis* sp. nov., *Acetobacter orleanensis* (Henneberg 1906) comb. nov., *Acetobacter lovaniensis* (Frateur 1950) comb. nov., and *Acetobacter estunensis* (Carr 1958) comb. nov. J Gen Appl Microbiol. 2000;46:147–65. http://dx.doi.org/10.2323/jgam.46.147
2. Sengun IY, Karabiyikli S. Importance of acetic acid bacteria in food industry. Food Control. 2011;22:647–56. http://dx.doi.org/10.1016/j.foodcont.2010.11.008
3. Snyder RW, Ruhe J, Kobrin S, Wasserstein A, Doline C, Nachamkin I, et al. *Asaia bogorensis* peritonitis identified by 16S ribosomal RNA sequence analysis in a patient receiving peritoneal dialysis. Am J Kidney Dis. 2004;44:e15–7. http://dx.doi.org/10.1053/j.ajkd.2004.04.042
4. Greenberg DE, Shoffner AR, Zelazny AM, Fenster ME, Zarember KA, Stock F, et al. Recurrent *Granulibacter bethesdensis* infections and chronic granulomatous disease. Emerg Infect Dis. 2010;16:1341–8. http://dx.doi.org/10.3201/eid1609.091800
5. Alauzet C, Teyssier C, Jumas-Bilak E, Gouby A, Chiron R, Rabaud C, et al. *Gluconobacter* as well as *Asaia* species, newly emerging opportunistic human pathogens among acetic acid bacteria. J Clin Microbiol. 2010;48:3935–42. http://dx.doi.org/10.1128/JCM.00767-10
6. Tuuminen T, Heinäsmäki T, Kerttula T. First report of bacteremia by *Asaia bogorensis*, in a patient with a history of intravenous-drug abuse. J Clin Microbiol. 2006;44:3048–50. http://dx.doi.org/10.1128/JCM.00521-06
7. Abdel-Haq N, Savaşan S, Davis M, Asmar BI, Painter T, Salimnia H. *Asaia lannaensis* bloodstream infection in a child with cancer and bone marrow transplantation. J Med Microbiol. 2009;58:974–6. http://dx.doi.org/10.1099/jmm.0.008722-0
8. Juretschko S, Beavers-May TK, Stovall SH. Nosocomial infection with *Asaia lannensis* in two paediatric patients with idiopathic dilated cardiomyopathy. J Med Microbiol. 2010;59:848–52. http://dx.doi.org/10.1099/jmm.0.019067-0
9. Gouby A, Teyssier C, Vecina F, Marchandin H, Granolleras C, Zorgniotti I, et al. *Acetobacter cibinongensis* bacteremia in human. Emerg Infect Dis. 2007;13:784–5. http://dx.doi.org/10.3201/eid1305.060532
10. Bittar F, Reynaud-Gaubert M, Thomas P, Boniface S, Raoult D, Rolain JM. *Acetobacter indonesiensis* pneumonia after lung transplant. Emerg Infect Dis. 2008;14:997–8. http://dx.doi.org/10.3201/eid1406.071236

Address for correspondence: Rebekka Kohlmann, Department of Medical Microbiology, Ruhr-Universität Bochum, Universitätsstraße 150, 44801 Bochum, Germany; email: rebekka.kohlmann@rub.de

Autochthonous Chikungunya Fever in Traveler Returning to Japan from Cuba

Motoyuki Tsuboi, Satoshi Kutsuna, Yasuyuki Kato, Eri Nakayama, Ken-ichi Shibasaki, Shigeru Tajima, Tomohiko Takasaki, Yuichi Katanami, Kei Yamamoto, Nozomi Takeshita, Kayoko Hayakawa, Shuzo Kanagawa, Norio Ohmagari

Author affiliations: National Center for Global Health and Medicine, Tokyo, Japan (M. Tsuboi, S. Kutsuna, Y. Kato, Y. Katanami, K. Yamamoto, N. Takeshita, K. Hayakawa, S. Kanagawa, N. Ohmagari); National Institute of Infectious Diseases, Tokyo (E. Nakayama, K.-i. Shibasaki, S. Tajima, T. Takasaki)

To the Editor: Chikungunya fever is a febrile illness caused by mosquito-transmitted chikungunya virus (CHIKV; genus *Alphavirus*, family *Togaviridae*). Clinical signs and symptoms typically begin with high-grade fever after an incubation period of 2–4 days (*1*). Other common symptoms include polyarthralgia, which is usually symmetric and involves multiple and distal joints, and skin involvement manifesting as a macular or maculopapular rash (*2*). Peripheral lymphadenopathy (most often cervical) and conjunctivitis might also occur (*3*).

Since late 2013, several outbreaks of illness caused by CHIKV have occurred in the Americas, including South America, the Caribbean, and the United States, which are outside this virus's former distribution area (*3*). Although autochthonous transmission of chikungunya fever has been reported in most Caribbean islands, only imported cases have been previously reported in Cuba (*4*). As increased numbers of US tourists visit Cuba after improved diplomatic relations in July 2015, reports of chikungunya fever cases in Cuba are of interest for travelers and healthcare providers. We describe a case of autochthonous chikungunya fever in a man who had traveled from Japan to Cuba.

In late February 2016, a previously healthy 27-year-old man visited a travel clinic in the National Center for Global Health and Medicine (Tokyo, Japan) with fever and rash. In mid-February, he had traveled to Havana and Santiago de Cuba in Cuba by way of Toronto, Ontario, Canada, for 11 days of sightseeing. He used no insect repellent during the trip and was unaware of any mosquito bites. When he sought care, he reported a high-grade fever (39°C) for 24 hours and several symptoms since the day of his return: retro-orbital pain, malaise, congested conjunctivas, and a

Acetobacter indonesiensis Bacteremia in Child with Metachromatic Leukodystrophy

Technical Appendix

Supplementary Patient Data

The patient was a 9-year-old child with late-infantile metachromatic leukodystrophy (MLD). MLD is an autosomal recessive, inherited, lysosomal storage disease, caused by deficiency of the enzyme arylsulfatase A (*1–3*). It involves accumulation of sulfatides in the nervous system, leading to progressive degeneration of myelin sheaths. In the most common late-infantile form, affected children exhibit developmental regression at the age of 12–24 months (*2,3*). Symptoms include muscle weakness and loss of previously acquired motor skills with progression to spastic tetraplegia, compromised vision and hearing, dysarthria, dysphagia, impaired breathing, incontinence, seizures, and dementia. Thus, MLD leads to progressive neurologic disability and dependency on nursing care, which ends in a decerebrate state and eventually death within 5–10 years after onset of symptoms. In addition, children with MLD may be considered prone to infections. They often die of pneumonia because of impaired breathing and dysphagia/aspiration (*2,3*). Also, healthcare- and device-associated infections may evolve while the patient is dependent on nursing care; for example, catheter-related bloodstream infections are common when the patient has intravascular devices (*4,5*). Treatment of MLD is limited to management of pain and symptoms (*1,3*).

When the patient was admitted to the hospital, she exhibited an advanced state of MLD. She was bedridden, had a tracheostomy tube for intermittent mechanical ventilation support, suctioning and bronchodilator/corticosteroid inhalation therapy, a percutaneous endoscopic gastrostomy feeding tube with a jejunal extension for enteral nutrition, and an implanted port catheter for application of drugs and parenteral nutrition in case of vomiting, which precluded application via the percutaneous endoscopic gastrostomy feeding tube. Because of gastroesophageal reflux and recurrent vomiting, the port catheter was frequently used, and the

patient was admitted for elective fundoplication. She was treated with proton pump inhibitors and antiemetics. In addition, she received muscle relaxants, anticonvulsants, and analgesics because of spasticity and seizures. In the past, she had suffered from recurrent pneumonia, with persistent *Pseudomonas aeruginosa* and *Enterobacteriaceae* colonization of the lung and tracheostomy tube. In the previous 2 months, she had, in addition, experienced recurrent *Candida tropicalis* bloodstream infection, which had been treated with caspofungin and port catheter removal with new port implantation.

As described in the letter, fever and increased C-reactive protein levels developed in the patient on hospital day 3 (Technical Appendix Table 1), most likely because of a port catheter–related bloodstream infection. Further laboratory parameters were normal, apart from a previously known and probably unspecific isolated elevation of gamma-glutamyltransferase (not shown), and physical examination and further diagnostic investigations (e.g., chest x-ray film) revealed no other focus of infection. *Acetobacter indonesiensis* was isolated not only from the blood culture drawn on day 3 from the port but also from the control blood culture drawn on day 10, even though the patient clinically responded to piperacillin/tazobactam and caspofungin treatment (Technical Appendix Table 1). Because of pathogen persistence, a port catheter exchange was recommended, but it was not performed because the patient's discharge was advanced to an earlier time, according to the parents' wish and against medical advice. The initially planned fundoplication was not conducted because of the advanced MLD after discussion of the patient case at the Clinical Ethics Committee.

Results of Microbiological Analyses

The blood culture drawn from the port catheter on day 3 of the hospital stay flagged positive after 72 hours of incubation. Gram stain revealed pleomorphic, mostly gram-negative rods, and subculture at 37°C and 5% CO_2 yielded growth of very small colonies on Columbia sheep blood agar and chocolate agar, but not on MacConkey agar (Technical Appendix Figure 1).

Species identification of the catalase-positive, oxidase-negative bacteria could not be achieved using matrix-assisted laser desorption/ionization (MALDI-TOF) mass spectrometry. Thus, partial sequencing of the 16S rRNA gene was performed by using primers established by

the Bund/Länder-Arbeitsgemeinschaft Gentechnik (LAG), Germany (16S-fw: GAA GAG TTT GAT CAT GGC TCA G; 16S-rev: ACG ACA GCC ATG CAG CAC CT). The sequence of our isolate has been deposited in GenBank (under accession no. KU976968), and it matched those of *A. indonesiensis* isolates deposited in the GenBank database (99.8% identity). 16S rDNA-based phylogeny confirmed affiliation to the species *A. indonesiensis* (Technical Appendix Figure 2).

Antimicrobial susceptibility testing was done by disk diffusion; devices for automated susceptibility testing were not used because of the slow pathogen growth. Zones of inhibition were merely detected for imipenem, meropenem, fosfomycin, and tigecycline (Technical Appendix Table 2). Interpretation of results (susceptible/intermediate/resistant) was not done because breakpoints for *Acetobacter* spp. are not available in the recommendations of the European Committee on Antimicrobial Susceptibility Testing (EUCAST).

References

1. Biffi A, Lucchini G, Rovelli A, Sessa M. Metachromatic leukodystrophy: an overview of current and prospective treatments. Bone Marrow Transplant. 2008;42(Suppl 2):S2–6. PubMed http://dx.doi.org/10.1038/bmt.2008.275

2. Fluharty AL. Arylsulfatase A Deficiency. In: Pagon RA, Adam MP, Ardinger HH, Wallace SE, Amemiya A, Bean LJH, et al., editors. GeneReviews® [Internet]. Seattle (WA): University of Washington, Seattle; 1993-2015.; 2006 May 30 [updated 2014 Feb 6].

3. van Rappard DF, Boelens JJ, Wolf NI. Metachromatic leukodystrophy: Disease spectrum and approaches for treatment. Best Pract Res Clin Endocrinol Metab. 2015;29:261–73. PubMed http://dx.doi.org/10.1016/j.beem.2014.10.001

4. Mermel LA, Allon M, Bouza E, Craven DE, Flynn P, O'Grady NP, et al. Clinical practice guidelines for the diagnosis and management of intravascular catheter-related infection: 2009 Update by the Infectious Diseases Society of America. Clin Infect Dis. 2009;49:1–45. PubMed http://dx.doi.org/10.1086/599376

5. Mirijello A, Impagnatiello M, Zaccone V, Ventura G, Pompa L, Addolorato G, et al.; Internal Medicine Sepsis Study Group. Catheter-related bloodstream infections by opportunistic pathogens in immunocompromised hosts. Eur Rev Med Pharmacol Sci. 2015;19:2440–5. PubMed

6. Flandrois JP, Perrière G, Gouy M. leBIBIQBPP: a set of databases and a webtool for automatic phylogenetic analysis of prokaryotic sequences. BMC Bioinformatics. 2015;16:251. PubMed http://dx.doi.org/10.1186/s12859-015-0692-z

7. Gouy M, Guindon S, Gascuel O. SeaView version 4: A multiplatform graphical user interface for sequence alignment and phylogenetic tree building. Mol Biol Evol. 2010;27:221–4. PubMed http://dx.doi.org/10.1093/molbev/msp259

Technical Appendix Table 1. Selected results of blood laboratory testing and temperature measurement at admission (day 1), at blood culture positivity (days 3 and 10) and a few days before discharge (day 14)*

| Parameter (unit)	Reference	Patient's result		
		Day 1	Day 3 (drawn from port)	Day 10 (drawn from port)	Day 14 (drawn peripherally)
Blood culture	Neg.	NA	Pos.	Pos.	Neg.
Body temperature (°C)	<37.0	36.1	39.5 ↑	39.0 ↑	37.3
Leucocytes (10^3/μL)	4.8–12.0	12.4	5.4	12.6	12.1
C-reactive protein (mg/dL)	<0.5	0.1	30.5 ↑	4.6 ↑	0.5

*N/A, not applicable; neg., negative; pos., positive; ↑, increased.
Pathologic/changed values are marked by arrows.

Technical Appendix Table 2. Results of antimicrobial susceptibility testing done by disk diffusion

Antimicrobial agent	Disk content (μg)	Zone diameter (mm)
Ampicillin	10	6
Piperacillin/tazobactam	30/6	6
Cefotaxime	5	6
Ceftazidime	10	6
Imipenem	10	18
Meropenem	10	12
Ertapenem	10	6
Fosfomycin/glucose 6-phosphate	50/50	19
Gentamicin	10	6
Trimethoprim/sulfamethoxazole	1.25/23.75	6
Tigecycline	15	15
Ciprofloxacin	5	6
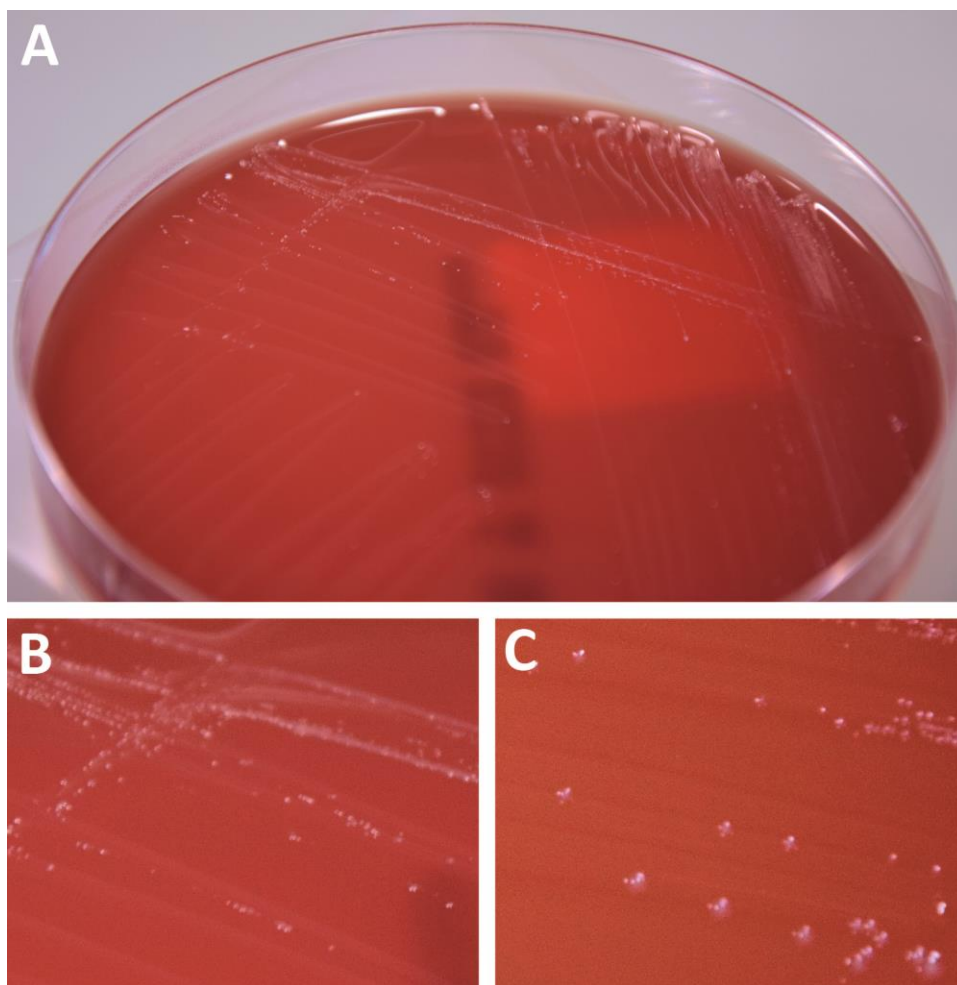
Technical Appendix Figure 1. *Acetobacter indonesiensis* grown on Columbia agar with 5% sheep blood after 48 h incubation (A). For better demonstration of colony morphologic features, we show 2.5-fold enlarged image sections of photos taken after 2 days (B) and 6 days (C).
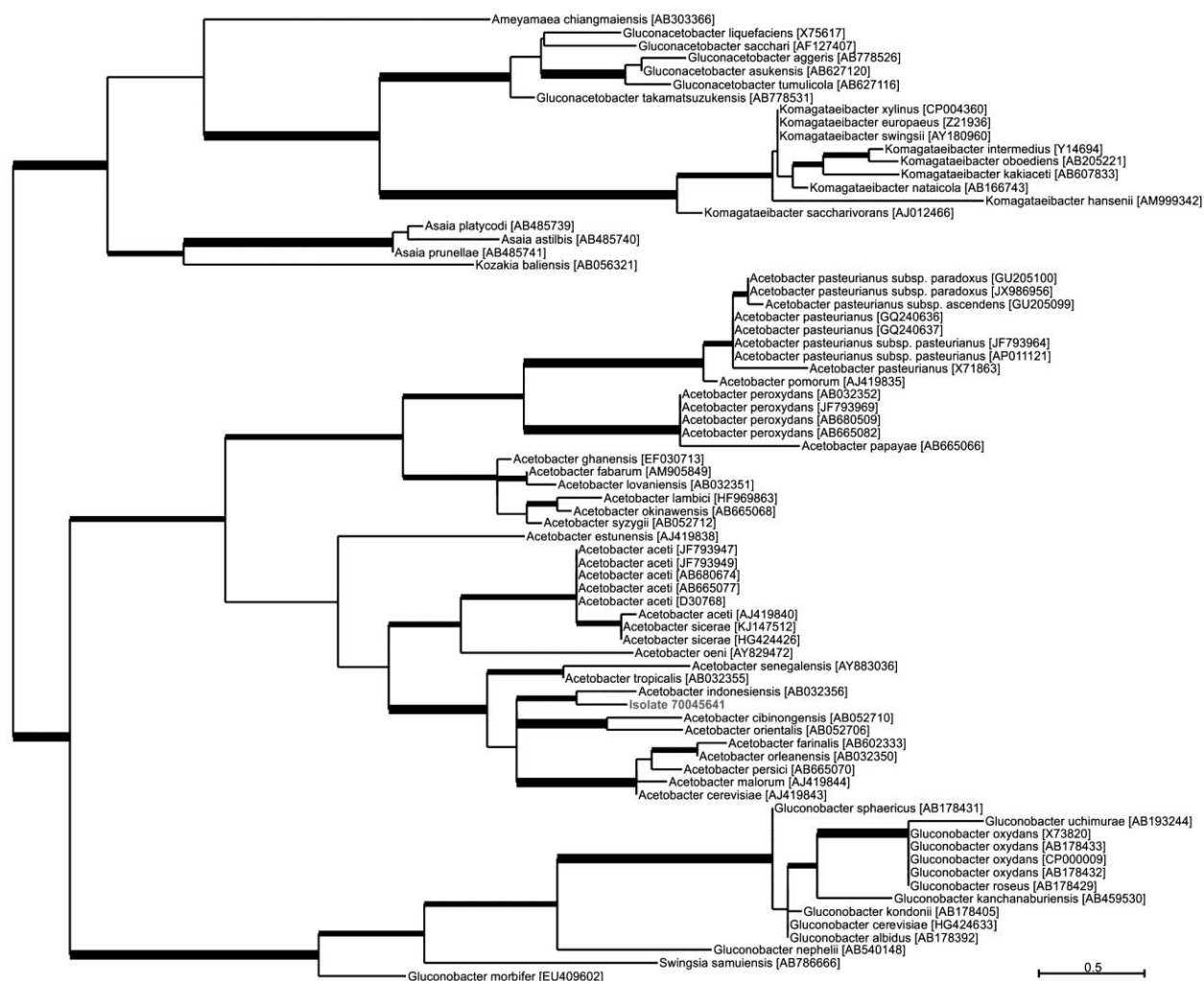
Technical Appendix Figure 2. Phylogenetic tree showing the position of our *Acetobacter indonesiensis* isolate within the genus *Acetobacter* and other genera of acetic acid bacteria. The tree was constructed by using the publicly available leBIBI^QBPP program which is based on 16S rDNA comparison by a maximum-likelihood approach (*6*). Our isolate 70045641 is highlighted in red. The 16S rDNA sequences used for comparison were obtained from the GenBank database by using the superstringent algorithm of leBIBI^QBPP, and accession numbers are indicated in brackets. The leBIBI^QBPP program uses the "branch width as support" option of SEAVIEW (*7*); the support is evaluated by Shimodaira-Hasegawa(SH)-like computation, and the largest width corresponds to SH>0.95 and can be considered as statistically significant, whereas the minimal line width (plain-line) is used when SH ≤ 0.80 and in this case the support is not sufficient. Scale bar indicates nucleotide substitutions per site.